

# THE MATURATION AND DISCHARGE OF ASCOSPORES OF THE APPLE SCAB FUNGUS IN INDIANA AND ITS SIGNIFICANCE IN CONTROL OF SCAB. R.C.BAINES.

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#### SUMMARY AND CONCLUSIONS

- 1. Scab ascospores matured before or parallel to the green-tip stage of apple buds in Indiana during the eight years from 1933 to 1940. It appears reasonable to predict, on the basis of the findings of this study, that mature ascospores will be present each spring when young leaves appear on the apple trees. Thus, apple growers in the areas studied may safely assume that ascospores will be mature when new growth appears on the trees in the spring, and that ascospores will be discharged during rainy periods from that time until sometime after the calyx stage. This situation does not appear to justify annual studies of spore maturity as a routine procedure to guide the timing of sprays.
- 2. Ascospores matured first in southern Indiana and progressively later in the northern counties.
- 3. The date on which ascospores matured varied from year to year and evidently was affected by climatic conditions.
- 4. Ascospores were discharged over a period of eight or more weeks. The percentage of ascospores discharged by the "calyx" stage of fruit development depended upon the frequency and duration of the rains and the time required for the apple trees to pass through the early stages of growth.
- 5. The location in an orchard in which leaves were overwintered appeared to have little effect on the time that ascospores mature in the spring.
- 6. There was no evidence of differences between the dates that ascospores matured in leaves of 11 varieties of apple that would have any practical significance.

# THE MATURATION AND DISCHARGE OF ASCOSPORES OF THE APPLE SCAB FUNGUS IN INDIANA AND ITS SIGNIFICANCE IN CONTROL OF SCAB

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The results of an eight-year study of maturation and discharge of ascospores of the apple scab fungus in Indiana orchards are presented in this bulletin. The study was initiated to provide information on the relationship between the time that ascospores mature in different latitudes in Indiana and the beginning of growth of apple trees, and to determine the value of providing such information on an annual basis for the guidance of growers in timing sprays. During the period 1934 to 1940, information on the maturity of the scab ascospores each spring was released to growers through the Agricultural Extension Service.

## Primary Scab Inoculum in the Spring

Ascospores, which are the primary inoculum of apple scab, are produced in dead apple leaves on the ground each spring. They are formed only in those leaves which were infected by the scab fungus prior to leaf-fall. The ascospores are formed in thin-walled sacs, or asci. within small, flask-shaped bodies called perithecia (figs. 1 and 2). The perithecia are sometimes visible as minute specks on the dead leaves. Mature ascospores are oblong, two-celled, and light brown as seen through the miscroscope. They are forcefully discharged into the air in the spring during rains when the leaves are moistened. Ascospores may be discharged four minutes after the leaves are wet, but usually a longer period of wetting is required.

Frequently ascospores are produced in large numbers and cause severe infection of the apple leaves, blossoms, and fruit. Keitt and Jones (4) determined the concentration of ascospores in the air in orchards during periods of discharge in Wisconsin. At one time they recorded an average of 289 ascospores per cubic foot of air during a four-hour period. Only a small percentage of the ascospores alight on susceptible apple parts and produce scab. However, the difficulty in controlling scab appears to be proportional to the numbers of ascospores discharged in an orchard. Scab may be controlled easily in orchards in which there is little scab inoculum, but usually unsatisfactorily controlled when abundant ascospores are produced in overwintering leaves in orchards, and when weather conditions are favorable for discharge of ascospores and infec-

# Methods of Over-Wintering and Sampling of Leaves

Scab-infected leaves of Delicious, Rome Beauty, Winesap, and Winter Banana varieties were collected near Lafayette and Martinsville, Indiana, during the latter part of October. They were mixed; then 300 to 400 leaves

<sup>&</sup>lt;sup>1</sup> The writer acknowledges the excellent cooperation of the following orchardists in sending to the Agricultural Experiment Station samples of over-wintered apple leaves used in this study: D. P. Bessire, A. L. Buchanan, Ferd Bolten, E. R. Bridges, V. V. Clark, Homer Coffing, L. V. Doud, Carl Garwood, Harold S. Hall, H. G. Hall, Robert Hubbard, Fred Lauman, Elvin Reinking, L. W. Runkle, Simpson Orchard Company, M. S. Troth, Wm. Walton (deceased), Albert H. Weil, John Zutt, and the assistance of James Tate and John R. Kurtz in examining the perithecia in the leaves.

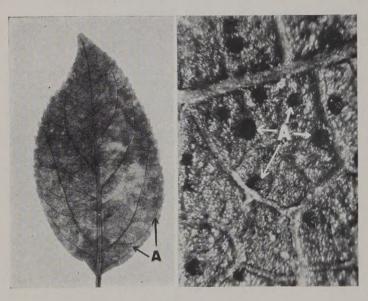
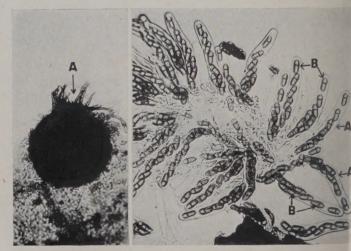


Fig. 1. Photographs of perithecia of the apple scab fungus in a partially cleared apple leaf. Numerous, small, dark perithecia visible as specks at "A" on the apple leaf on the left. Right: An area of the apple leaf magnified 50 times and showing the perithecia protruding from the surface at "A".

Fig. 2. Photographs of perithecia and of ascospores of the apple scab fungus. Left: Single perithecium partly removed from leaf tissue. The perithecium is round and dark and has a thick wall. It contains ascospores, which are discharged through a small opening near the top at "A". Magnified 250 times. Right: The ascospores, eight in each ascus, or sac, were squeezed from a mature perithecium. The thin wall of the ascus is visible at "A" and the two-celled ascospores at "B". Magnified 430 times.



were placed on well-drained sod areas and covered with pieces of inch-mesh, woven, galvanized wire, about three feet square. In 1933, leaves were overwintered in orchards near Bedford, Lafayette, and Orleans, and in the following seven years near Aurora, Bedford, Bloomfield, Bristol, Covington, Denver, Edinburg, Evansville, Lafavette, LaPorte, Linton, Logansport, Nashville, Moores Hill, Orleans, Seymour, and Vincennes, Indiana (fig. 3). At weekly intervals during March and April, 15 to 20 of the over-wintered leaves were mailed to the Agricultural Experiment Station by the cooperating fruit growers. (See footnote 1, page 1.) Apple buds in the early stages of development also were enclosed.

To facilitate examination, the leaves were cleared by heating in five percent sodium hydroxide, washed in water, and placed in 95 percent alcohol. Five to ten leaves bearing perithecia were selected from each sample, and between 20 and 30 of the largest perithecia were removed from each leaf and examined under a microscope. The perithecia were classified as containing small asci, large asci, small spores, immature lightcolored spores, and dark or mature spores, and by the percentage of spores discharged. The most advanced stage of development of the asci and spores in each perithecium was recorded. The number of asci which had discharged spores was estimated, since it would have been difficult and excessively timeconsuming to determine the exact numbers of asci which had discharged spores. When perithecia were crushed on a glass slide, the empty distended asci which had discharged their spores were readily distinguished from asci which had not yet discharged their spores.

To verify the assumption that darkcolored ascospores would be discharged when moistened, leaves bearing mature perithecia were moistened, placed in moist chambers, and allowed to discharge ascospores onto glass slides. The slides then were examined and the number of discharged ascospores recorded.

#### Time of Ascospore Maturity

The dates on which ascospores matured and Delicious trees reached the green-tip stage of bud growth are presented in table 1, for the period 1933 to 1940. For convenience in comparing the time that the ascospores matured in different locations in the state, the state has been divided into five areas as designated in fig. 3. Ascospores matured between 2 and 38 days in advance of the green-tip stage of the apple buds in all cases except two. In



Fig. 3. Locations, designated by "X", where apple leaves were placed for overwintering in five areas of Indiana.

the central area in 1936, and in the north-central area in 1939, ascospores did not mature until Delicious trees were at the green-tip stage. Ascospores matured between 18 and 25 days in advance of the green-tip stage of the apple buds in all areas of Indiana in 1938. In that year ascospores were discharged in many areas prior to the appearance of young leaves.

Ascospores matured earliest in southern Indiana and usually matured progressively later from the south to the

Table 1. Dates When the First Ascospores of the Apple Scab Fungus Matured and of Green-tip Stage of Apple Buds in Areas of Indiana, from 1933 to 1940

	Sou	Southern	South	South-central	Cen	Central	North	North-central	Nor	Northern
Year	Spores	Green-tip stage1	Spores	Green-tip stage	Spores	Green-tip stage	Spores	Green-tip stage	Spores	Green-tip stage
1933			Mar. 9	Mar. 30			Mar. 3	Apr. 10		
934	Mar. 22	Apr. 1	Apr. 22	Apr. 6	Mar. 26	Apr. 8	Mar. 29	Apr. 10	Apr. 10	Apr: 19
935	Mar. 16	Mar. 20	Mar. 15	Mar. 22	Mar. 20	Mar. 26	Mar. 27	Apr. 1	Apr. 2	Apr. 10
936	Mar. 12	Mar. 26	Mar. 16	Mar. 28	Apr. 62	Apr. 5	Mar. 31	Apr. 8	Apr. 10	Apr. 12
1937	Mar. 10	Mar. 25	Mar. 15	Mar. 28	Apr. 5	Apr. 14	Apr. 12	Apr. 16	Apr. 16	Apr. 20
1938	Feb28	Mar. 18	Mar. 1	Mar. 20	Mar. 3	Mar. 23	Mar. 2	Mar. 26	Mar. 11	Apr. 5
939	Mar. 20	Mar. 27	Mar. 20	Mar. 30	Mar. 25	Apr. 2	Apr. 3	Apr. 3	Apr. 13	Apr. 20
1940	Mar. 18	Mar. 30	Mar. 20	Apr. 3	Mar. 26	Apr. 6	Apr. 9	Apr. 15	Apr. 10	Apr. 25

<sup>1</sup> Green-tip stage of Delicious buds.
<sup>2</sup> First sample received.

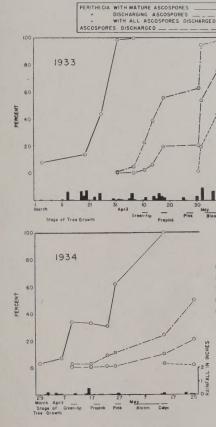
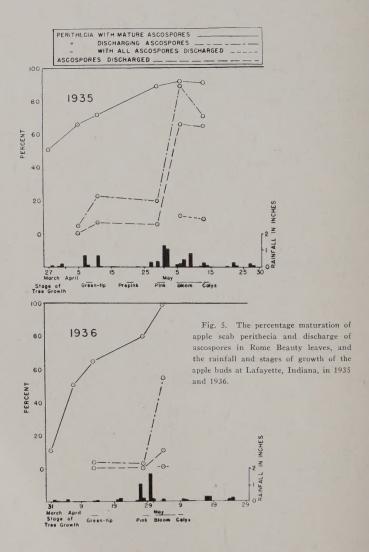


Fig. 4. The percentage maturation of apple scab perithecia and discharge of ascospores in Rome Beauty leaves, and the rainfall and stages of growth of the apple buds at Lafayette, Indiana, in 1933 and 1934.



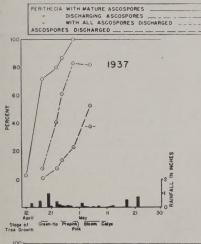
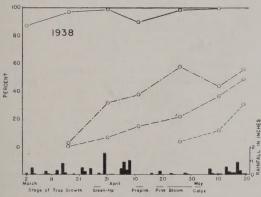


Fig. 6. The percentage maturation of apple scab perithecia and discharge of ascospores in Rome Beauty leaves, and the rainfall and stages of growth of the apple buds at Lafayette, Indiana, in 1937 and 1938.



north. The spread between the dates that the first ascospores matured in southern and northern Indiana varied from 11 days in 1938 to 37 days in 1937. The differences in the time that the first ascospores matured in the various parts of the state are likely due to climatic factors. Thus, the average mean temperature from November 1, 1936, to February 30, 1937, was 8.5°F. higher at Evansville in the southern area than at Goshen in the northern area.

The number of ascospores discharged during the early stages of growth of the apple trees has an important bearing on the control of scab. The data presented in figs. 4 to 6 show the progressive development and discharge of scab ascospores during the early stages of growth of the apple trees at Lafayette, Indiana, for 1933 to 1938. The amount and distribution of rainfall and the stage of growth of the apple buds also are shown. For the years 1933 to 1938 respectively, approximately 100, 34, 70, 65, 72, and 98 percent of the perithecia contained some asci with mature ascospores at the green-tip stage of the apple buds, while 82, 10, 65, 11, 53, and 28 percent of the ascospores had been discharged at the calyx stage, respectively, for the same years.

The percentage of ascospores discharged during the precalyx period, when the young apple parts are very susceptible to scab infection, depends chiefly on the frequency and duration of rains and the maturity of the ascospores. On April 1, 1933, 98 percent of the perithecia contained some asci with mature ascospores, and a few ascospores had been discharged. Frequent rains occurred during April and May of 1933, which caused 82 percent of the ascospores to be discharged by May 22, one week after the calyx stage. During April and May of 1934 and 1936, few periods of rain conducive to discharge of ascospores occurred, with the result that only 10 and 11 percent, respectively, of the ascospores were discharged by the calyx stage. During the time between the green-tip and calyx stages in 1935, nine rainy periods occurred, and during the same growth period in 1937, there were six rainy periods during which ascospores probably were discharged. At the calvx stage in 1935, 65 percent, and in 1937, 53 percent of the ascospores had been discharged. In 1938, ascospores matured early, and seven rains conducive to discharge of ascospores and infection occurred during the period between the green-tip and calyx stages. At the calyx stage 28 percent of the ascospores had been discharged, and during the 18-day period after the calyx stage, 21 percent of the ascospores were discharged.

Ascospores were discharged after the calyx stage at Lafayette during each of the eight years. Leaves from other sections of Indiana also showed that as a rule discharges of ascospores occur after the calvx stage. Adams (1) reported that ascospores are discharged during May and June in Delaware, and Schneiderhan and Fromme (5) found that ascospores may be discharged as late as July in Virginia. In Wisconsin, Keitt and Jones (4) found that ascospores were discharged during April, May, and June. Hamilton (3) in New York reported that ascospores were discharged during May and June in 1932, and during April, May, and June in 1933 and 1934.

#### Maturation of Ascospores in Leaves Over-wintered at Different Sites in an Orchard at Bedford

Apple leaves were over-wintered at different sites in the Purdue Agricultural Experiment Station Orchard at Bedford to determine whether certain conditions in the orchard affected earliness of ascospore maturation topography of this orchard is rolling, with a number of deep drainage-draws leading to sink holes. Leaves were over-wintered in low areas, on slopes of different exposures, and on high Rome Beauty and Winesap leaves infected with scab were collected on October 25, 1933, and over-wintered beneath wire screen at eight sites in this orchard. Rome Beauty and Stayman Winesap leaves were placed at only four of the sites on November 9, 1934, and on November 7, 1935.

In 1934, ascospores matured relatively late. Mature ascospores were found in leaves over-wintered at six of the eight sites on April 4. However, no difference in the maturity of the ascospores appeared in the leaves from the different sites examined on April 14 and 30. Approximately 11 percent of the perithecia in the leaves at all of the sites contained some asci with mature ascospores on April 14. On April 30, 28 percent of the perithecia examined contained some asci with mature ascospores, and six percent of the perithecia had discharged ascospores. One percent of the potential number of ascospores had been discharged by April 30.

No significant differences in perithecial development were found in the leaves over-wintered at the four sites in 1935 and in 1936. The over-wintered leaves contained mature ascospores on March 18, 1935. Approximately nine percent of the ascospores had been discharged on March 27, and 50 percent on April 11. Ascospores in leaves at

the four sites were nearly mature on March 11, 1936, and mature on March 26.

### The Development of Ascospores in Varieties of Apple Leaves

The time of maturity and discharge of ascospores in 11 varieties of apple leaves was determined at Lafayette in 1938. Leaves infected with scab were picked from the trees on November 2, 1937, and were enclosed in envelopes made of half-inch mesh hardware cloth and placed on sod for over-wintering. In the spring, from 15 to 30 perithecia were removed from each of five leaves of each variety, and the maturity and percentage of ascospores discharged were determined.

Between 1 and 30 percent of the perithecia from all of the varieties, except Delicious and Maiden Blush, contained some mature ascospores on March 2, 1938 (table 2). On March 17, between 33 and 98 percent of the perithecia in the 11 varieties of leaves contained mature ascospores. Ascospores had been discharged from leaves

Table 2. Maturation of Perithecia and Discharge of Ascospores in Eleven
Varieties of Apple Leaves at Lafayette in 1938

	Percen	Percentag		
Variety	Mature ascospores		Some ascospores discharged	of ascospores discharged
1	(Mar. 2)		(April 11)	(April 11)
Benoni	1	75	64	24
Black Ben Davis	15	36	31	4
Delicious	0	33		
Maiden Blush	01	57	71	46
Mann	5	98	50	25
Rome Beauty	8	67	40	20
Gallia Beauty	21	74	40	20
Stayman Winesap	12	67	21	7
Turley	30	72	30	20
Winter Banana	15	82	15	3
York Imperial	. 8	78	22	9
Average	10	67	35	16

<sup>1</sup> Fourteen percent of the perithecia contained nearly mature (light-colored) ascospores.

of ten varieties on April 11. The differences in the percentages of mature and of discharged ascospores in the leaves of the 11 varieties are not believed to be of practical significance, since differences as great as those recorded between the varieties in table 2 frequently occurred among leaves of the same variety. Also, the maturation of ascospores in the Delicious leaves possibly are not typical of this variety, since very few perithecia occurred in the Delicious leaves in 1938, while in 1939 numerous perithecia were formed, and the ascospores matured as early as in 11 other varieties. Wilson (6) found that ascospores matured earlier in leaves of a number of varieties of crab apples than in the leaves of some commercial varieties.

#### Discussion and Application

The amount of ascosporic inoculum in orchards affects the ease of control of scab and usually is proportional to the amount of infection which developed on the leaves the previous year. Thus, there is a carry-over effect of one year's control or lack of control to the next year. Severe scab infection which frequently develops on the foliage, especially of susceptible varieties, leads to the formation of abundant scab perithecia and ascospores in overwintered leaves. The results of unpublished spray tests and observations show that it is difficult to control scab when abundant ascospores are formed in the orchard and the weather is comducive for the discharge of ascospores and infection.

Young apple leaves, blossoms, and fruit are very susceptible to scab in-

fection from the time that they first appear until shortly after the calvx stage. They should be protected from infection by thorough and timely applications of fungicides through this period. During this early period of development the young apple leaves, blossom buds, and fruit increase rapidly in area, and frequently it is difficult to maintain on them a protective coverage of spray which is adequate to prevent scab infection. Baines and Benedict (2) showed that Rome Beauty leaves on blossom spurs grew to nine times their initial area between the prepink and calvx stages in 1934. Under most conditions in Indiana apple trees should be protected by sprays applied at 7 to 10-day intervals during the prepink and calyx stages. More frequent applications of sprays may be necessary during periods of frequent rains, which remove some spray deposit from the trees and also favor the discharge of ascospores and infection.

In orchards which contain little inoculum, fungicides for the control of scab on somewhat resistant varieties such as Jonathan, Grimes Golden and Stayman Winesap may be safely omitted after the calyx spray. However, on the extremely scab-susceptible varieties such as Rome Beauty, Mc-Intosh, Winesap and Delicious, abundant scab infection by ascospores and by secondary spores (conidia) frequently develops before and after the calyx stage. Therefore, on such scab-susceptible varieties, a fungicide should be used in the first cover spray when weather conditions have been favorable for scab infection during the precalyx period and especially when frequent rains occur after the calyx stage.

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